Performance of a Full-Scale Sidestream DEMON® Deammonification Installation
Andrea Nifong¹, Andy Nelson², Chandler Johnson³, Charles B. Bott⁴
¹Civil and Environment Engineering Department, Old Dominion University, Norfolk, VA 23529
²Hampton Roads Sanitation District, 515 Back Creek Rd., Seaford, VA 23462
³World Water Works, Inc, 4000 SW 113th Street, Oklahoma City, OK 73173
⁴Hampton Roads Sanitation District, 1436 Air Rail Ave., Virginia Beach, VA 23455

ABSTRACT
Anaerobically digested sludge dewatering liquors (e.g. centrate) can represent 15-25% of the TKN load on a typical municipal WWTP. Sidestream nitrogen removal has been demonstrated to be an effective tool for improving nitrogen removal performance and reliability with a savings in aeration energy, chemicals (supplemental alkalinity and carbon), and sludge production.

Deammonification, partial nitritation by ammonia oxidizing bacteria (AOB) combined with anaerobic ammonium oxidation (AMX), provides near complete nitrogen removal with a 65% reduction in energy and 100% reduction in supplemental carbon and alkalinity requirements as compared to traditional nitrification-denitrification. The DEMON® treatment process is one available sidestream deammonification treatment process which is a single step deammonification system in which both partial nitritation and AMX occur in the same tank, operated similar to a typical sequencing batch reactor (SBR).

The process is anticipated to decrease supplemental alkalinity (sodium hydroxide), carbon (methanol), and aeration energy usage at the 15 MGD HRSD York River (YR) treatment plant, which currently includes a fully aerobic nitrifying activated sludge system with post denitrification filters. The DEMON® was installed in existing SBR tanks that were part of a decommissioned water reuse system. DEMON® installation at York River is a first in North America, while over 30 full-scale systems are currently operated in Western Europe.

The DEMON has reached stable 80% ammonia removal to date and provided insight to establish startup procedures for successful and efficient DEMON startups in North America.

KEYWORDS
DEMON, deammonification, anammox

INTRODUCTION
Ammonia is one of the primary pollutants of concern for the wastewater treatment industry. Millions of dollars are spent annually on ammonia removal alone. As populations grow, water consumption increases and regulations become more stringent, the need for more economical and efficient technologies has become one primary objective for wastewater treatment research.
**Conventional Nitrification/Denitrification.** Historically, nitrogen removal technologies have focused on optimizing the conventional nitrification and denitrification pathways of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB).

**Nitrification:**

\[
\begin{align*}
NH_4^+ + 2O_2 & \rightarrow NO_3^- + 2H^+ + H_2O \\
2NO_2^- + O_2 & \rightarrow 2NO_3^- 
\end{align*}
\]

AOB first oxidize NH$_4^+$ to NO$_2^-$ then NOB oxidize NO$_2^-$ to NO$_3^-$. These ammonia removal processes are often coupled with heterotrophic denitrification to provide total nitrogen removal. Based on the stoichiometry above, the oxygen required for complete oxidation of NH$_4^+$ is 4.57 g O$_2$/gN oxidized. 3.43 g O$_2$/g N is used for nitrite production and 1.14 g O$_2$/oxidized to nitrate.

Nitrification also consumes 7.4 g of alkalinity as CaCO$_3$ per the following reaction:

\[
NH_4^+ + 2HCO_3^- + 2O_2 \rightarrow NO_3^- + 2CO_2 + 3H_2O
\]

The consumption values above (Metcalf&Eddy 2003) can be used to estimate the costs associated with operating a conventional nitrification process and provide a good foundation for the comparison of resources used in this process versus alternative ammonia oxidation processes such as the DEMON.

In anoxic conditions, heterotrophic bacteria reduce nitrate to nitrite and then to dinitrogen gas which is released to the atmosphere completing the total nitrogen removal process. Most of these bacteria are facultative aerobic organisms which can use oxygen as well nitrogen species as the electron acceptor. In the absence of oxygen, nitrate or nitrite is used as the electron acceptor. The need for an exogenous carbon source is dependent both on where along the treatment train denitrification takes place and the wastewater characteristics. Pre-anoxic denitrification precedes nitrification, in this case the organic substrate in the influent wastewater typically provides the electron donor for oxidation reduction. When the denitrification process follows nitrification it is termed post-anoxic denitrification which requires the addition of an external carbon source to increase the reaction rate of the process. This is often the case in polishing filters such as those located at the York River plant. The complete denitrification reaction with a typical wastewater as the carbon source and methanol as a carbon source is shown below. (Metcalf&Eddy 2003)

**Wastewater:**

\[
C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \]

**Methanol:**

\[
5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \]

Denitrification produces 3.57 g of alkalinity as CaCO$_3$ per g of NO$_3^-$-N reduced.

Conventional nitrification/denitrification is resource intensive, requiring large treatment tanks and supplemental chemicals for optimization. Conventional nitrification/denitrification has been used as a sidestream treatment process, however, it is not ideal. These processes require high COD loadings not typically present in sidestream wastewaters.
**Sidestream Treatment Alternatives.** The development of processes which utilize nitrite shunt such as SHARON (single reactor for high activity ammonia removal over nitrite) have been developed for sidestream treatment alternatives. These processes aim to stop the nitrification pathway at the oxidation of NO$_2^-$ or rather perform nitritation and then reduce NO$_2^-$ to N$_2$ via denitrification. There are a variety of ‘short cut’ treatment processes that work well in sidestream treatment such as SHARON,

Nitrite shunt pathways typically save 25% in aeration, 40% in carbon, and 40% reduction in biomass over conventional nitrification/denitrification (Hellinga 1998).

Nitrite shunt process provides an ideal ratio of NH$_4^+$ : NO$_2^-$ and typically used as a first stage in two-step deammonification processes such as the OLAND and SHARON-Anammox (Cema, 2006; Pynaert, 2002).

**Sidestream Deammonification.** Since its discovery in 1999 (Strous et al. 1999; Mulder et al., 1995; van der Graaf 1996), the deammonification process has been successfully applied to sidestream centrate treatment. Deammonification is a two step process which utilizes the symbiotic relationship of two genera of bacteria, ammonia oxidizing bacteria (AOB) and anaerobic ammonia oxidizers (AMX).

Half of the NH$_4^+$ load is oxidized to NO$_2^-$ by AOBs (nitritation). The remaining NH$_4^+$ and the produced NO$_2^-$ is then reduced to N$_2$ by ammonia oxidizing bacteria called anammox. The basic energy reaction and complete reaction for cell synthesis are given below (Neethling 2012).

$$\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + \text{H}_2\text{O}$$

(6)

$$1.0\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.66\text{HC}_3\text{O}_4^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.66\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 2.03\text{H}_2\text{O}$$

(7)

The above reaction determined by Strous et al (1999) is universally accepted in the literature and provides the stoichiometric ratios accepted to indicate the presence of deammonification by anammox. For every mole of NH$_4$-N, 1.32 moles of NO$_2$-N are consumed and 0.26 moles of NO$_3^-$-N are produced. Additionally, the anammox reaction provides alkalinity as 0.13 moles of H$^+$ are consumed.

Sidestream deammonification processes provide approximately 63% reduction in required $O_2$, nearly 100% less carbon, 80% less biomass, and typically requires no additional alkalinity over conventional nitrification/denitrification. Sidestream deammonification can be performed in a variety of reactor configurations. One reactor configurations such as the DEMON and CANON (Constantine 2005) are one-step deammonification processes, while two-reactor configurations are two-step processes. Both one-step and two-step processes provide the same resource savings.
Table 1. Resource Savings of alternative sidestream treatment options compared to conventional nitrification/denitrification

<table>
<thead>
<tr>
<th>Process</th>
<th>$O_2$</th>
<th>Carbon Demand (as COD)</th>
<th>Alkalinity</th>
<th>Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite Shunt</td>
<td>25%</td>
<td>40%</td>
<td>0</td>
<td>40%</td>
</tr>
<tr>
<td>Deamination with AMX</td>
<td>63%</td>
<td>~100%</td>
<td>~100%</td>
<td>80%</td>
</tr>
</tbody>
</table>

Although the resources saved make DEMON an attractive treatment process, the anammox maximum specific growth rates are slower than AOB which typically are 1 to 1.2 day$^{-1}$ (Anthonisen et al., 1976). Anammox growth rates have been reported as 0.05 to 0.2 day$^{-1}$ (Strous et al., 1999; Tsushima et al., 2007; Van der Star et al., 2008). The slow growth rates of AMX require enrichment prior to the start-up of the system which has been the primary difficulty in bringing this technology to North America. Due to the inability to harvest AMX biomass, the first DEMON treatment system took 2.5 years to accumulate sufficient biomass for the full scale design (Wett 2006). After the establishment of this system, enrichment could be continued and biomass harvested for rapid startup of other full scale installations.

**York River Facility Overview.** Screening, aerated grit removal tanks, a pre-aeration tank along with an odor control system and three primary clarification tanks comprise the headworks and primary treatment. Primary clarified effluent is routed to a conventional fully nitrifying step feed activated sludge process for secondary treatment and then to three secondary clarifiers. Secondary clarifier effluent is pumped to denitrification filters which were part of the 2011 upgrades at York River. The nine deep bed filters were sized for build out (30 MGD) and include a methanol feed system and backwash air blowers and pumps. After denitrification and chlorination/dechlorination, the effluent is discharged to the York River.

The solids handling facility receives both primary and waste activated sludge into gravity thickening and dissolved air flotation, respectively. There are two anaerobic digesters and solids are dewatered by centrifuge.

A full-scale DEMON® process was installed at the Hampton Roads Sanitation District’s (HRSD) 15 MGD York River Treatment Plant to treat centrifuge centrate. The DEMON treatment technology was a perfect fit for HRSD’s York River Plant. The plant recently terminated operation of a tertiary treatment SBR system which had been used to provide reuse water to a neighboring industry. The available SBR tanks made the DEMON treatment system a low capital cost side stream treatment option.

**Objectives.** Since York River was the first DEMON installation in North America it provided an opportunity to document the startup and optimization of the treatment process. As part of this effort, the following objectives for the installation are outlined:

**OBJECTIVE 1:** Establish harvesting and transportation procedures for AMX biomass from Europe in order to enrich AMX in North America.

**OBJECTIVE 2:** Establish start-up and monitoring procedures for the DEMON process. Optimize operating strategies.
OBJECTIVE 3: Identify any possible operation issues that DEMON installations may experience and develop solutions that can be applied to rectify or prevent them.

![York River centrate equalization tank (left) and DEMON tank (right)](image)

**Figure 1.** York River centrate equalization tank (left) and DEMON tank (right)

**METHODS AND MATERIAL**

*Bench Scale Measurements.* Two batch reactors were used during activity measurement testing. The beakers were made of polypropylene and had a maximum volume of 5L with a working volume of 3L. A gang mixer with paddle impellers was used for mixing both reactors. A diffuser stone was installed in both reactors to provide air/oxygen or nitrogen for aerobic, anoxic, or anaerobic conditions. For anoxic and anaerobic experiments a tight fitting Styrofoam lid was used to cover the biomass with inlets for the mixer blade extension, DO probe, and pH probe. Dissolve oxygen (DO) was held close to constant using YSI 58 dissolve oxygen meter with a YSI 5739 field oxygen probe. pH was monitored using Nova Analytics Corporation Pinnacle pH electrode and automatically controlled using acid (1M $\text{H}_2\text{SO}_4$) and sodium hydroxide (1 M NaOH). The temperature was held constant by placing the reactors in a Fischer Scientific Isotemp water bath. Refer to Figure 2 and for images of the batch reactor setup.
AOB and NOB activity were measured in 3L batch scale reactors by monitoring NO₂⁻-N and NO₃⁻-N production rate, respectively. Samples were collected for 2.5 hours at 30-minute intervals. Dissolved oxygen (DO) was maintained between 1.0-2.0 mg/L. pH was maintained at 7.5. Samples were immediately filtered through 0.45 membrane filters and analyzed.

For NOB rate measurements, 20 mg/L NO₂⁻N was spiked into the mixed liquor. Samples were tested for NO₃⁻-N, NO₂⁻-N, and NH₄⁻-N concentrations. NOB activity was determined from the NO₃⁻-N production rate.

AMX activity was measured in 3L batch scale reactors by monitoring NH₃-N and NO₂⁻-N production over time. Samples were collected for 3 or 4 hours at 30-minute intervals. Anoxic conditions were achieved by sparging with nitrogen with atmospheric CO₂ balance. After initial sparging the nitrogen supply was terminated and the reactor maintained zero DO concentrations throughout the test period. pH was maintained at 7.5. An initial NO₂⁻ concentration of 50 mg/L NO₂⁻N was used in the test. NO₂⁻N was fed as necessary during the test to prevent limiting conditions. Immediately after collection the samples were filtered through 0.45 μm filters. AMX activity was determined from NH₄⁻-N and NO₂⁻-N consumption and NO₃⁻-N production, respectively.

Performance Monitoring. Samples for on-site performance monitoring and batch reactor experimentation were collected at the following locations. Influent samples were collected upstream at a sample valve located on the outlet piping of the equalization tank. DEMON process samples were collected at a sample port located on the influent of the cyclone manifold. Effluent samples were collected from the effluent decant line. The samples were collected and immediately filtered with a .45 micron glass fiber filter membrane.
Process monitoring was also performed by the HRSD Central Environmental Laboratory (CEL). These grab samples were collected from three sample locations. The influent and effluent sampling locations were the same as above, process samples were collected as a grab sample from DEMON tank.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Centrate Influent</th>
<th>DEMON Process</th>
<th>Centrate Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>TVSS</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>COD</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>sCOD</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>TKN</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>NH3-N</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>NOX-N</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>NO2-N</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>TP</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>OP</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

Process NO$_3^-$-N, NO$_2^-$-N, and NH$_4^-$-N, and OP concentrations were analyzed daily in order to evaluate operational performance. These analytes were measured using various HACH colorimetric test kits and a HACH DR2800 spectrophotometer. The methods and procedures used to monitor daily concentrations are shown in the table below, all analysis conducted by the HRSD CEL laboratory were performed in accordance with Standard Methods and procedures.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method Title</th>
<th>Reference Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthophosphate</td>
<td>Ascorbic Acid Method</td>
<td>EPA 365.1</td>
</tr>
<tr>
<td>Ammonia (NH3-N)</td>
<td>Salicylate Method</td>
<td>EPA 350.1, EPA 351.1, EPA 351.2</td>
</tr>
<tr>
<td>Nitrate (NO3-N)</td>
<td>Dimethylphenol Method</td>
<td>40 CFR 141</td>
</tr>
<tr>
<td>Nitrite (NO2-N)</td>
<td>Diazotization Method</td>
<td>141EPA 353.2</td>
</tr>
</tbody>
</table>

**DEMON Process Control.** The DEMON process is controlled by a PLC program developed by the pH control strategy of Dr. Bernhard Wett (Wett 2007). York River’s SBR cycle time is 8 hours, processing three cycles per day. Each cycle is comprised of intervals of aeration, feeding with anoxic mixing, and anoxic mixing periods. The length of each of these intervals can be controlled in two ways; timers or pH setpoints. Both high pH and low pH setpoints are available to provide robust control. This capability allows the user to determine the exact amount of ammonia conversion desired. Once the desired ammonia:nitrite ratio is achieved and the low pH setpoint is reached, the controller will terminate aeration. During the anoxic period, centrate is fed. The addition of raw centrate along with the anammox oxidation of NH$_4^+$ taking place provides ALK, resulting in a slight pH increase. Centrate also provide sCOD which may be utilized by heterotrophic denitrifying bacteria during the anoxic interval to reduce NO$_3^-$-N. The system will remain anoxic until the high pH setpoint or the defined set time interval is reached.
These intervals of aeration and anoxic periods continue until the controller enters into sedimentation and decant. This program control allows for simple instrumentation requiring only pH and DO to control the entire program. Blowers are controlled by a PID loop which is set to maintain a target DO concentration.

**Instrumentation.** The PLC receives signals from three sensors and various flow rate and level transmitters. pH, DO, and temperature can be viewed in the trending screens shown below. The pH increase during anoxic periods (AMX active) and the pH decrease during aeration intervals (nitritation) is clearly evident in Figure 3. This clear pH fluctuation is indicative of a well performing system as well as a direct visual representation of the process performance. During nitration, alkalinity is consumed and pH decreases. During anoxic periods, AMX activity produces alkalinity, driving the pH up, and centrate feeding during anoxia provides additional alkalinity. If this pH fluctuation is not observed, substrate, DO, or alkalinity concentrations may be insufficient, or inhibitory substances may be present.

![Figure 3. pH trend screen](image)

<table>
<thead>
<tr>
<th>Trend</th>
<th>Value</th>
<th>Date/Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demon_1_O2</td>
<td>-0.037516</td>
<td>5/20/2013 9:08:03.471 AM</td>
</tr>
<tr>
<td>Demon_1_FH</td>
<td>6.777199</td>
<td>5/20/2013 9:08:03.471 AM</td>
</tr>
</tbody>
</table>
The conductivity signal is provided as a trending tool. Trending conductivity provides an easy and effective way to evaluate the overall efficiency of the process in terms of NH₄⁺ removal and NO₂⁻ and NO₃⁻ production. An increase in conductivity may be due to nitrite accumulation caused by over aeration or some other problem. Regardless, a simple look at the conductivity data can indicate whether the process is operating efficiently.

All probes used for the DEMON operational control are stable and reliable requiring infrequent calibration and/or maintenance. NH₄⁺, NO₂⁻, or NO₃⁻ sensors, unlike pH and DO sensors, typically require regular maintenance and calibration for accurate measurement and control.

The DO concentration is controlled by a PID loop. The target concentration was kept between 0.3 and 0.5 mg/L. Initially the DO probe was located with pH and conductivity probes along the tank wall mounted on an instrument float. After attempts to tune the PID which resulted in DO spikes which could not be mitigated through tuning, DO profile testing was performed in various locations throughout the tank. The results indicated that due to the mixing conditions inside the tank...
tank, the side walls of the tank were experiencing DO plumes of high and low concentration (due to tank swirling) which were impeding DO controller robustness. In response, the instrument float was relocated into the center of the tank which does not experience such severe DO concentration fluctuations, and the PID was re-tuned.

Two DO concentration targets were input into the PLC throughout startup. Both 0.5 mg/L and 0.3 mg/L. An appropriate target DO concentration is a topic of controversy. Although it has been readily reported that low DO concentrations preferentially select for AOB over NOB (Huang et al. 2010; Starkenburg, 2007; Henze, 2000), recent research indicates exactly the opposite may be the case for mainstream conditions (Regmi, 2012). Most DEMON processes are operated at a setpoint of 0.3 mg/L DO.

More data are needed to determine the optimum target dissolved oxygen concentration.

**Biomass Separation Device (BSD).** A biomass separation device was installed on a manifold to control floc-phase SRT, Figure 6.

The biomass separation device drives the formation of and separates anammox-dominant granules from flocs and returns the anammox granules back to the process through the underflow while sending the floc fraction to waste. Optimizing cyclone run time for solids wasting is critical. During initial startup only AMX biomass was present thus the BSD was not operated. Once nitrate was present in the system, in excess of 13% NO₃⁻ production/ NH₄ removed, the BSD was used for solids wasting. The system is operated between a 4 to 6 day floc-phase SRT. If NO₃⁻ production increases above acceptable values (~13% production) cyclone run time should be increased to waste excess NOB.
Design Criteria.

Table 4. Design Criteria

<table>
<thead>
<tr>
<th>Centrate Feed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN</td>
<td>600-1,000 mg/L</td>
</tr>
<tr>
<td>Flow (7 day equalized flow)</td>
<td>71,500 gpd</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN</td>
</tr>
<tr>
<td>NH₄⁺</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floc Phase SRT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQ Tank</td>
</tr>
<tr>
<td>DEMON Tank (min)</td>
</tr>
<tr>
<td>DEMON Tank (max)</td>
</tr>
</tbody>
</table>

Centrate Dewatering Strategy. Numerous operational strategies have been tested to determine the most stable operational strategy for both the DEMON process and the solids handling facility. Approximately 122,000 gpd are processed through the centrifuge and sent to the centrate equalization tank which is approximately 71,500 gpd over a 7-day equalized flow.

The digesters have been operated both in parallel and series; series configuration being typical operation. However due to digester cover replacement and maintenance, single digester operation and reduced volatile solids destruction was experienced during two periods since startup. The effect of operational digester configuration on the ammonia load is significant. Ammonia concentrations in single digester mode are typically 400-500 mg/L while influent NH₄⁺ concentration when operated in series is typically 900-1000 mg/L. The effect this loading differential has had on the efficiency of the DEMON system has not yet been quantified, however, data is continually being collected to determine this.

Prior to the DEMON system, dewatering took place 4 to 5 days a week as a batch process (approximately 8 hours). Bringing the DEMON system online, required the consideration of the stability of another biological process. Therefore a variety of dewatering schedules were attempted until one was found which achieved the best balance for both processes and the operators. Dewatering currently takes place 5 days a week over a 24 hour period.

RESULTS AND DISCUSSION

Start Up. The DEMON system was seeded October 10th, 2012 with 5,000 gallons of concentrated (5% solids) sludge by mixing with plant effluent and pumping into the DEMON. The seed biomass was shipped from Europe and was dormant for approximately one and half months prior to seeding. On October 12th, the system was fed centrate to achieve an ammonium concentration of approximately 50 mg-N/L. The SBR cycling was initiated, feeding the system at 20% total capacity and reaching 50% capacity within the first four weeks of operation. Specific load was then increased no more than 10% a week, and aerobic/anoxic time intervals adjusted as needed.
Figure 7. Aerobic/Anoxic Time Intervals per Ammonia Loading

As shown in Figure 7, the anoxic time length was slowly decreased throughout startup while the aerobic time length was increased. This trend held true except for the initial startup when aerobic time was purposely increased to promote the growth of AOB.

System Optimization. The York River installation has provided lessons learned for all to consider when installing a DEMON system. The process has experienced stable periods along with specific operating issues which have been resolved. The primary monitoring parameters are shown below; ammonia removal shown in red is the primary indicator of the efficiency of the system. The correlation between system performance and temperature is very evident (Figure 8). Nitrite accumulation is both an indicator of AOB activity and AMX activity shown in blue while nitrate production is the primary indicator of NOB prevalence.

The system was shut down once in late April due to a malfunctioning actuator on the effluent decant valve. The system was down for four days, meaning it was anoxic and mixing. Immediately once the system was started up again, AMX and AOB activity resumed at a slightly reduced load (10% reduction).
**Nitrite Accumulation.** The DEMON control system is designed to maintain an appropriate DO set-point to convert half of the ammonia load to nitrite. The DO requirement can be calculated from ammonia load and the oxygen transfer efficiency (OTE). This requirement was calculated for a variety of ammonia loads and water depths.

The oxygen demand required for nitritation can be calculated based on the target ammonia removal efficiency and stoichiometric requirements of the deammonification process. 90% ammonia removal is the target process efficiency.

Nitritation requires \(3.43 \frac{mg \text{ } O_2}{mg \text{ } NH_4-N}\) according to the following oxidation reaction (Metcalf & Eddy, 2003):

\[
2NH_4^+ + 3O_2 \leftrightarrow 2NO_2^- + 4H^+ + 2H_2O \tag{8}
\]

However, AOB’s only convert 56.9% of the \(NH_4^+\) removed to \(NO_2^-\), so the above \(O_2\) demand must be modified to take this into consideration. The 56.9% conversion is accounted for by the analysis of the following ratios (Wett, 2006) developed from the stoichiometric anammox equation (8).

\[
\frac{0.26 \text{ } mol \text{ } NO_3^-}{1 \text{ } mol \text{ } NH_4^+} \tag{9}
\]

\[
\frac{1.32 \text{ } mol \text{ } NO_2^-}{1 \text{ } mol \text{ } NH_4^+} \tag{10}
\]
Using the above assumptions, $O_2$ demand can be calculated for any given influent NH$_4$\textsuperscript{+} load. Furthermore, taking into account oxygen transfer efficiency of the diffusers, the airflow requirement along with the exact blower speed input can be calculated for any given ammonia load and control settings.

This calculation can be used to ensure that excess dissolved oxygen is not being supplied to the process, for example due to a faulty signal from the DO sensor. Excessive dissolved oxygen concentrations will lead to AMX inhibition indicated by nitrite accumulation. AMX activity has been documented to decline when exposed to increasing dissolved oxygen concentrations (Strous and Kuenen, 1999; Ward et al., 2011). In this scenario, NOB’s can easily flourish, having both dissolved oxygen and nitrite substrates readily available and reduced competition for the nitrite from AMX. Once NOBs become prevalent, it is difficult to induce their decline.

However, during startup the activity of the AOB population was minimal due to the extended anoxic conditions and thus dissolved oxygen concentrations were slowly increased over short time increments. After a couple of days of this operation with no increase in nitrite the dissolved oxygen supply was drastically increased and AOB activity quickly became prevalent. At these high oxygen levels, AMX is reversibly inhibited. This inhibition was evident by the accumulation of nitrite (up to 30 mg/L). In response the feed and aeration was terminated and the tank was allowed to mix anoxically until the nitrite concentrations were below 7 mg/L (less than 48 hours).

Nitrite accumulated in the tank one other time due to excessive DO levels. This was caused by the air blast system wearing a hole in the LDO sensor cap, a common occurrence with the HACH LDO air blast system. The hole in the LDO sensor cap caused irregular readings in the DO and thus excess DO was supplied to the tank. After a period of anoxic mixing, the nitrite levels returned to acceptable levels. To help lengthen the life of the LDO sensor cap the air blast timer was modified to blast the cap at longer intervals and the blast piece was modified to provide a longer distance between the air blast and the sensor cap.

**Temperature.** The DEMON system was started in early October with average temperatures of 23 °C. Optimal operating temperatures of deammonification processes have been reported between 35-40 °C (Dosta et al., 2008; Strous and Kuenen, 1999; Toh et al., 2002) while significant activity losses have been reported below 15 °C (Dosta et al. 2008). Thus starting the DEMON system in cooler temperatures was not ideal, and lower removal efficiencies were anticipated.

As ambient air temperatures declined through the month of October, ammonia removal efficiencies also declined as seen in Figure 8. The efficiency decline lagged the temperature by a few days but shows close correlation. This correlation between temperature and removal efficiency prompted additional upgrades to the DEMON system in an attempt to increase the temperature of the centrate.

Even though centrate temperature consistently averages 35 °C leaving the digesters, the centrate lost significant heat during pumping from the digester to the equalization tank (800 feet) and then during equalization, since the stainless steel tank is uninsulated and above-ground. There was a 5 °C drop from the equalization tank to the DEMON which could not be improved. Thus effort was focused on increasing the temperature in the equalization tank. Construction delays
prevented a summer startup, which might have alleviated the need for additional heat during this period.

On November 28, 2012, a 1.5 MBTU boiler and heat exchanger was installed for temporary heating of the equalization tank. Immediate increase in temperature can be seen in Figure 8 from the installation. Operation of the boiler was lost twice after installation, and the effects of this temperature loss directly reduced ammonia removal efficiency of the system. The boiler was set to maintain a temperature of 35°C in the equalization tank, consistently keeping a temperature of 30°C in the DEMON process tank.

January 24, 2013 additional upgrades were made to improve heat retention of the centrate equalization tank. A floating ball blanket was added to the tank to cover the water surface as seen below in Figure 9. These hollow plastic balls provide a thermal insulation barrier between the air/liquid interface thus aiding in heat retention.

![Figure 9. Centrate equilization ball blanket](image)

**Nitrate Accumulation.** After four months of stable performance, ammonia removal efficiency declined from 70-80% to 50-60% (Figure 8). The cause of this decline was unknown, since no changes in temperature or operating inputs were made. This ammonia removal decline was correlated with an increase in NOB activity. Nitrate production increased from 13-20% to 30-50% NO$_3^-$/NH$_4^+$ removed. Additionally, nitrite production declined. The lack of AOB activity along with the increase in NOB activity severely increased competition for the AMX thus leading to a drop in ammonia removal.

Deammonification theoretically produces 11.2% NO$_3^-$, and any additional NO$_3^-$ production can be attributed to increased NOB activity, typically 13% is the operational target.

Two possible causes were identified and simultaneously evaluated (1) micronutrient deficiency and (2) enrichment of NOB in the cyclone underflow. It is also possible that these two causes are related or one may have induced the other. As such the identification the definitive cause of the increase in NOB activity and decrease in AOB activity is not known, however, it can be assumed that it was a combination of both of the identified issues.
Micronutrient Deficiency. Micronutrients are essential for the successful activity and growth of bacteria (Grady and Daigger 2011). However, the exact quantity of various elements required is disputed depending on the source and bacterial community under evaluation. Generally, there are sufficient quantities of these required micronutrients in municipal wastewater, however, this is not always the case in sludge dewatering liquors.

In activated sludge processes the presence or deficit of micronutrients can shift the predominance of bacteria species (Ribbons, 1970). Since species attempt to adapt to the availability of micronutrients, bacteria which can adapt to lower concentrations will become predominant (Ribbons, 1970; Grady and Daigger, 2011). The DEMON system started to exhibit a shift in bacteria populations towards a larger prevalence of NOBs. It may be possible that they either require less of a certain micronutrient than AOB and/or AMX or that their synthesis process allows for easier utilization of these nutrients, however, optimum rate measurements of AMX, AOB, and NOB activity have not indicated any limitation in AMX activity at any point during operation (Figure 10).

![Figure10. AMX activity measurements](image)

Trace elements could be removed from the wastewater stream from the following dominant mechanism: adsorption, hydroxide precipitation, and/or hydrogen sulfide precipitation (Ribbons, 1970). Since centrate has been through the entire plant treatment processes, there are numerous occasions for trace elements to be removed prior to the centrate reaching the DEMON process. This is especially probable since polymer is added prior to anaerobic digestion which may bind micronutrients into forms that are not accessible to microorganisms. Iron addition at York River for chemical P removal may also be precipitating trace metals in the digester.

The ammonia mono-oxygenase (AMO) enzyme is used by AOB to catalyze the oxidation of NH$_3$ to hydroxylamine (NH$_2$OH). NH$_2$OH is then oxidized to NO$_2^-$, by hydroxylamine oxidoreductase.
The oxidation of NH$_3$ to NO$_2^-$ is the source of energy for growth in AOB. Both copper and iron have been identified as key metal components for the function of the AMO enzyme. Copper requirements and toxicities have been studied for AOB, specifically the AMO enzyme. Ensign et al. (1993) studies identified copper requirements through the recovery of activity by the addition of copper. AMO activities increased 5 to 15 times when exposed to Cu additions (Ensign et al., 1993). Studies have indicated that copper is an important component of AMO and acts as a binuclear site on the enzyme during the oxidation of NH$_3$ (Shears and Wood, 1985).

To evaluate micronutrient concentrations, the approximate required values of micronutrients reported by Grady and Diagger (Table 5) were used to identify possible nutrient deficiencies.

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Approximate Requirements µg/mg Biomass COD Formed</th>
<th>Approximate Requirement µg/mg Biomass TSS Formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Calcium</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Magnesium</td>
<td>7</td>
<td>8.4</td>
</tr>
<tr>
<td>Sulfur</td>
<td>6</td>
<td>7.2</td>
</tr>
<tr>
<td>Sodium</td>
<td>3</td>
<td>3.6</td>
</tr>
<tr>
<td>Chloride</td>
<td>3</td>
<td>3.6</td>
</tr>
<tr>
<td>Iron</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.2</td>
<td>0.24</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Copper</td>
<td>0.02</td>
<td>0.024</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.004</td>
<td>0.005</td>
</tr>
<tr>
<td>Cobalt</td>
<td>&lt;0.0004</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

Resulting tests indicate that cobalt, copper, zinc, and potentially manganese and molybdenum may have all been insufficient for optimum performance.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>68.8 67.9</td>
<td>2.18 ND</td>
<td>ND</td>
</tr>
<tr>
<td>Calcium</td>
<td>80.7 78.2</td>
<td>31.9 85.9</td>
<td>73.7 31</td>
</tr>
<tr>
<td>Magnesium</td>
<td>16.5 16.8</td>
<td>2.26 14.2</td>
<td>14.1 2.37</td>
</tr>
<tr>
<td>Sodium</td>
<td>154 153</td>
<td>3.74 157</td>
<td>160 3.98</td>
</tr>
<tr>
<td>Iron</td>
<td>3.05 1.29</td>
<td>19.7 ND</td>
<td>ND</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.032 0.068</td>
<td>0.198 0.028</td>
<td>0.51 0.492</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.15 0.067</td>
<td>0.383 0.154</td>
<td>0.154 0.375</td>
</tr>
<tr>
<td>Copper</td>
<td>&lt;0.01 0.01</td>
<td>0.03 0.026</td>
<td>0.04 0.124</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.012 0.01</td>
<td>0.019 &lt;.02</td>
<td>&lt;.02 0.023</td>
</tr>
<tr>
<td>Cobalt</td>
<td>&lt;0.02 &lt;0.02</td>
<td>&lt;0.02 &lt;.02</td>
<td>&lt;.02 0.028</td>
</tr>
</tbody>
</table>

The DEMON has been receiving a mixture of micronutrients since the deficiencies were identified on April 2nd, 2013. The resulting improvement in ammonia removal was immediate. Within one day of adding the micronutrients ammonia removal efficiency increased 20%.
However stable improvement in ammonia removal was not immediate, but was achieved approximately 2 months after continued feeding of micronutrients. During these two months modifications to the cyclone operation were made. The resulting improvement in performance may be from a combination of both.

![Figure 11. Performance data after micronutrient addition.](image)

Figure 11 and 12 depict performance and activity changes. The black line indicates the start of micronutrient blend addition on April 2\textsuperscript{nd}. The grey line depicts a doubling of cyclone run time on April 14\textsuperscript{th}. The decrease in ammonia removal is most likely due to additional wasting of AOB biomass and a decrease in nitrite. The system was shut down and mixed anoxically from April 20\textsuperscript{th}-24\textsuperscript{th}, while maintenance was performed on the decant valve actuator. Once the system was brought back on line, performance continued to improve. Starting May 3\textsuperscript{rd} the cyclone operation strategy was adjusted depicted by the red line.

The effects of operational strategies are mirrored in the AOB/NOB activity measurements (Figure 12). Activities increased when the boiler was installed late in November. The NOB activity increased drastically when the LDO sensor cap failed and the PLC received faulty DO signals, causing excess DO supply to the system. Early in January, solid wasting was terminated for 5 days to increase activity. Once solid wasting was re-initiated the activity rates declined. NOB inhibition was not consistently achieved until micronutrient addition and cyclone shearing was maintained for two months. Although NOB activity is less than AOB activity during the month of March, ammonia removal declined during this time and nitrate production increased (Figure 11). The DEMON system has maintained consistent 80% ammonia removal and less than 20% NO\textsubscript{3}\textsuperscript{-} production for the last two months.
**Biomass Separation.** The biomass separation device of the DEMON are hydrocyclones. The York River DEMON utilizes four hydrocyclones on one manifold system to separate the SRT’s of the different bacteria populations. The AOB and NOB populations are typically cycled up the hydrocyclones and wasted while the AMX granules are cycled down through the underflow and returned to the process. This allows for a separation of the floc-phase SRT from the granule-phase SRT.

Typical floc-phase SRT durations are 2-10 days, depending on the influent characteristics and the temperature of the system. AMX SRT is not typically calculated but can be assumed to be in excess of 30-50 days based on the approximate efficiency of the cyclone for retaining granules.

Typically, the structure of the AOB and NOB populations are light flocs which are cycled out the overflow of the cyclones, however, rate measurements taken from the overflow, underflow, and process streams indicated that the AOB and NOB flocs were co-enriched in the underflow of the cyclones (Figure 11 and 12). This phenomenon is unique to York River and has not been identified in any other DEMON installation. This phenomenon coincided with increased NO₃⁻ concentrations and reduced NO₂⁻ accumulation, both primary indicators that NOB prevalence is too high for an optimized deammonification process.
Visual inspection and TSS data collected on the underflow, overflow, and process samples of the cyclone both indicated that more biomass was being returned to the process through the underflow than is typical for DEMON cyclone operation. In an effort to immediately supply additional shearing three of the cyclone overflows were diverted back into the process tank, leaving one to waste. This also required a quadrupling of the waste rate determined by the cyclone run time per cycle.

<table>
<thead>
<tr>
<th>Cyclone Sample</th>
<th>05/03/2013</th>
<th>05/06/2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underflow</td>
<td>10,500</td>
<td>7,210</td>
</tr>
<tr>
<td>Overflow</td>
<td>772</td>
<td>980</td>
</tr>
<tr>
<td>Process</td>
<td>2,000</td>
<td>2,960</td>
</tr>
</tbody>
</table>

Additional shearing did decrease biomass in the underflow and increase biomass in the overflow, however, not drastically (Table 7).

Why additional biomass was returned in the underflow may have been a combination of both micronutrient deficiency, affecting cell structure, and the amount of shear force in the tank. Steady state floc formation is a product of the given shear rate in a reactor given that floc size is created by the continuous balance between aggregation and breakage due to the shear force within the tank (Jarvis et al., 2005; Biggs, 2000).

Flocculation is the result of balancing floc accumulation and break up within the system. An ITT Flygt submersible mixer was installed in the DEMON process tank with a 159 mm (6.25 in.) impeller. The mixer is 4 hp with a max 855 rpm operating in 363 (95,921 gal) of process water. Imparting 8.6 $W/m^3$ with an approximate G value of 103 $s^{-1}$. It is possible that this mixer is providing insufficient shear to the biomass, but no granule settling problems have been observed. Regardless, a new larger mixer will soon be installed. The proposed Landia mixer is 9hp, impeller diameter of 558.8 mm (22 in.) with a max speed of 388 rpm. Imparting 19 $W/m^3$ with an approximate G value of 155$s^{-1}$. This mixer will be installed in late summer of 2013.

During the time the sludge was recirculated through the cyclones, rate measurements were performed on the cyclone underflow, overflow, and process streams to evaluate any changes in AOB and NOB activity in the respective process streams. The shearing was started on May 3rd and is indicated by the grey line on the graphs below.
Nitrospira ssp has been shown to be the stronger flocculating bacteria when compared to other AOB populations and in fact have been identified as growing in granules (Carvalho et al., 2006).

This is evident from the activity tests (Figure 13). Throughout the exposure period to high shear stress the AOB population were more reactive, the increase in activity is assumed to directly correlate to the increase in biomass flocs within each respective stream. The AOB population exhibited a more pronounced response to the shear stress; deflocculating easier than the NOB population and becoming more dominant in the overflow. The increase of NOB activity in the overflow is less pronounced indicating that the flocs are stronger. Regardless, AOB/NOB activity in the overflow and process has increased since shearing and micronutrient addition.
Figure 14. NOB:AOB activity ratio underflow, overflow, and process adjusted to 33°C

The entire contents of the tank have been exposed to high shear for over five weeks. Since that time ammonia removal efficiency has increased 20%. However, the results of these tests are inconclusive since the lack of micronutrients may have caused the change in floc structure and thus affecting the microbial community within the process streams.

CONCLUSIONS

The startup and optimization of the York River DEMON process has successfully demonstrated 80% ammonia removal to date and established operating and maintenance procedures. Additionally, possible issues have been identified and successfully solved.

Some of the conclusions are:

(1) A dominant NOB population can be repressed without the use of external inhibitors.

(2) The DEMON operational strategy provides robust control of the deammonification process through DO and pH control.

(3) Centrate characterization prior to startup should include micronutrient panels to ensure the presence of micronutrients required for successful AOB productivity.
REFERENCES


